FABRICATION AND CHARACTERIZATION OF A PERFUSION BIOREACTOR FOR INTERFACE TISSUE ENGINEERING

A THESIS SUBMITTED FOR PARTIAL FULFILLMENT

OF THE REQUIREMENT FOR THE DEGREE OF

BACHELOR OF TECHNOLOGY

IN

BIOMEDICAL ENGINEERING

By:

KUNDAN JOSHI

(Roll no. 109BM0007)

Under the guidance of:

Dr. BIBHUKALYAN PRASAD NAYAK

Department of Biotechnology and Medical Engineering,

National Institute of Technology, Rourkela

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CERTIFICATE

This is to certify that the project entitled, "**Fabrication and Characterization of a Perfusion Bioreactor for Interface Tissue Engineering**" submitted by **Kundan Joshi** is an authentic work carried out by him under my supervision and guidance for the partial fulfillment of the requirements for the award of **Bachelor of Technology (B. Tech) Degree in Biomedical Engineering** at **National Institute of Technology, Rourkela.**

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/ Institute for the award of any Degree or Diploma.

Date:

Place: Rourkela

Dr. Bibhukalyan Prasad Nayak

Dept. of Biotechnology & Medical Engineering

NIT Rourkela

ACKNOWLEDGEMENT

"KNOWLEDGE IS INCOMPLETE WITHOUT MOTIVATION & DIRECTION"

I would really like to take this opportunity to thank my project guide, **Dr. Bibhukalyan Prasad Nayak** for believing in me and allowing me to work on this project and motivating me throughout the time. I also owe a debt of gratitude to **Mr. Akshaya Kumar Padhi** for all the help I have got from him. This Final Year Project has brought out the best in me and I have given my everything to be able to live up to the expectations of all.

Apart from this, I would like to thank everyone in the Rehabilitative & Regenerative Medicine Laboratory for assisting and motivating me throughout my project.

Thank you, one and all

Kundan Joshi

B. Tech (Biomedical Engineering)

National Institute of Technology, Rourkela

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ABSTRACT:

Current trends in replacement of ligaments have had less success in terms of regenerating the Enthesis (Bone-Ligament or Bone-Tendon Interface) due to lack of Insertion Technology. A more assuring approach would be regenerating the ligament graft by Interface Tissue Engineering. There is a need of specialized multi-compartmental bioreactors to simulate the environment of enthesis. Such a bioreactor was designed and fabricated which would facilitate perfusion flow through a cell-seeded scaffold in a culture medium that resembles the fluid present in synovial joint cavity. The perfusion bioreactor design also incorporated independent setups for the simultaneous application of tension and compression as mechanical stimulus to the cell-scaffold construct.to facilitate the regeneration of enthesis. Accordingly the temperature, pressure, pH and dissolved oxygen concentration of the system can be regulated in-vitro to mimic the in-vivo conditions. Hence, the proposed perfusion bioreactor has the capability to simulate all the environmental parameters for proper development of a bone-ligament interface with enhanced insertion strength.

Keywords: Perfusion bioreactor, synovial joint, enthesis, bi-axial mechanical stimulus, insertion

CHAPTER 1

INTRODUCTION

1. INTRODUCTION:

1.1 BIOREACTOR

A bioreactor may refer to any manufactured or engineered device or system that supports a biologically active environment. In other words, a bioreactor effectively mimics a body-fluid environment so as to cater to the culturing of biologically active constituents. As for example, the medium in the bioreactor functions as the blood of the living beings so as to supply oxygen and essential nutrients to the culture. It can be used for various purposes such as aerobic/anaerobic chemical processes, fermentation, cell culture, tissue engineering applications as well as industrial purposes such as large-scale production of vaccines, antibodies, and bio-conversion.

FIGURE 2.1 *Bioreactor designed by United States Department of Energy*

(Image available on public Domain, not copyright)

1.2 BIOREACTOR COMPONENTS:

Bioreactor of different sizes and configurations are available. Different components of a bioreactor are listed below along with a schematic of the same:

i) Reactor Vessel/tank
ii) Agitator/Stirrer Motor
iii) Stirrer Shaft
iv) Air Sparger
v) Baffles
vi) Cooling Jacket
vii) Feed pump
viii) Feed vessel
ix) Air Outlet
x) Sensors

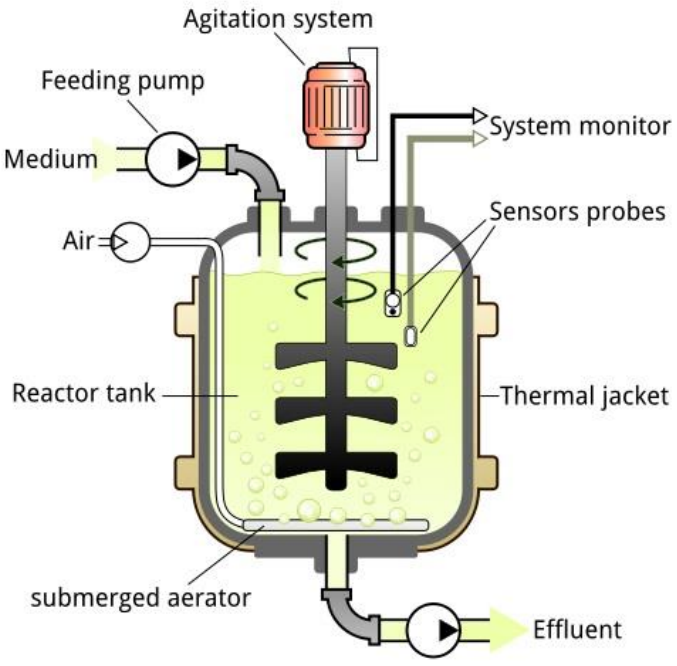

FIGURE 2.2 *Schematic showing basic components of a typical bioreactor*

i) Reactor Vessel:

Reactor Vessel acts as a container to provide an environment suitable for media and cells for the culture to proceed.

ii) Agitator/Stirrer Motor:

Stirrer Motor is required to mix the contents in the vessel for creating a homogeneous environment and also required to prevent the formation of vortex. It is generally a motor present at the bottom or top of the reactor vessel.

iii) Stirrer Shaft:

The major function of shaft is to stir and homogenize the whole media substrate mixture. It is connected to stirrer motor which gives it rotational energy.

iv) Air Sparger:

Sparger helps in air circulation. It is responsible for supplying the air into the reactor vessel so that aeration is properly maintained.

v) Baffles:

Baffles are pole like structures present generally in four in number in four opposite ends of the fed-batch reactor vessel. Baffles efficiently help to create turbulence in the media flow in the reactor vessel so that mixing of the substances is enhanced and formation of vortex in corners is prevented.

vi) Cooling Jacket:

It is a jacket that covers the reactor vessel and helps to maintain a constant temperature required for culture. Water flow in the cooling jacket generally helps in maintaining the temperature.

vii) Feed pump:

Feed pump is used to pump the feed input into the reactor. The time, amount and speed of the input feed can be easily specified with the help of feed pump.

viii) Feed vessel:

It is a container/vessel for feed input.

ix) Air Outlet:

It allows flow of effluent gases out of the reactor vessel. This is important when aeration is required.

x) Sensors:

i) pH probe – measure the pH of the solution.

ii) DO probe – measure the Dissolved Oxygen content.

iii) Temperature Sensor – measure temperature.

iv) Pressure Sensor – maintain air-tight condition.

v) CO_2 Sensor – measure Carbon dioxide content.

vi) Foam Sensor – measure Foaming and reduce foaming

1.3 BASICS OF SYNOVIAL JOINT CAVITY:

1.3.1 Synovial Joint

Synovial Joint is the most common and most movable type of joint in the human body. Synovial Joint is also known as diarthrosis and makes up most of the bones of the human body. Synovial joints achieve movement at the point of contact of the articulating bones.

The different types of synovial joints are gliding joint (carpals), hinge joint (knee, elbow), pivot joint (atlanto-axial joint), ellipsoid joint (radio-carpal joint), saddle joint (carpo-metacarpal joint) and ball & socket joint (shoulder, hip). There are some structural and functional characteristics of synovial joints which make them different from cartilaginous or fibrous joints.

1.3.2 Characteristics of Synovial Joint:

i) In Synovial Joint, there is existence of capsules surrounding the articulating surface of the joint and presence of lubricating synovial fluid within those capsules/synovial cavities.

ii) The articular surfaces are covered with a slippery layer of hyaline cartilage which promotes free movement.

iii) The joint is surrounded by an articular fibrous capsule which is sensitive to stretches imposed by movements because of the rich nerve supply to the fibrous capsule.

iv) Varying degrees of movements are always permitted by synovial joints.

1.3.3 Factors Affecting Stability of the synovial joint:

i) Shape of Articulating surfaces

ii) Structures like capsules, ligament

iii) Tone of the muscle

iv) Gravitational forces

v) Pressure(sub-atmospheric)

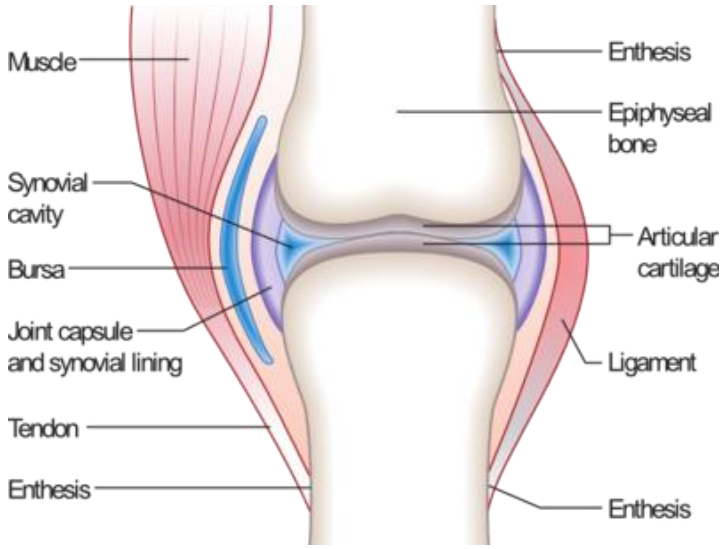

FIGURE 2.1 *Synovial Joint*

1.3.4 Synovial Fluid:

Synovial fluid is a viscous, non-Newtonian fluid found in the cavities of synovial joints. With its yolk-like consistency ("synovial" partially derives from ovum, Latin for egg), the principal role of synovial fluid is to reduce friction between the articular cartilage of synovial joints during movement. Synovial fluid is secreted by synovial membrane that is present in the inner membrane of synovial joints.

The fluid contains hyaluronic acid secreted by fibroblast-like cells in the synovial membrane and interstitial fluid filtered from the blood plasma. This fluid forms a thin layer at the surface of cartilage and also fills in the irregularities in the articular cartilage surface. The fluid in articular cartilage effectively serves as a synovial fluid reserve. During movement, the synovial fluid held in the cartilage is squeezed out mechanically to maintain a layer of fluid on the cartilage surface (so-called weeping lubrication).

The functions of the synovial fluid include:

i) Reduction of friction - synovial fluid lubricates the articulating joints
ii) As a dilatant fluid, it functions as shock-absorber.
iii) Synovial fluid becomes more viscous under applied pressure i.e. it becomes thick once shear is applied in order to preserve the joint and then, becomes thin to normal viscosity instantaneously so as to resume its lubricating function.
iv) It also functions as transporter of nutrient and waste. The fluid supplies oxygen as well as nutrients. It also removes carbon dioxide and metabolic wastes from the surrounding cartilage. It also contains phagocytic cells that remove foreign elements and debris that results from normal wear and tear in the joint.

1.4 PERFUSION BIOREACTOR:

A Brief History:

Initial approaches at cell culture and tissue engineering were accomplished by cell seeding in three-dimensional scaffolds and suspension culture. Suspension culture was done in either spinner-flask or rotating wall vessel motor bioreactors. In the former, the scaffold was hanged in the flask and media was dispersed using a magnetic stirrer. In the latter, the whole reactor vessel was rotated and along with it, the media and cells on the inside. But, to enhance mass-transfer rates in bioreactors, necessity of specialized bioreactors was felt.

Perfusion bioreactors are however, specialized bioreactors which incorporate perfusion flow to supply growth factors and essential nutrients to the cells seeded inside the pores of the scaffolds.

Perfused flow literally means flow over or through the system. Perfusion bioreactors have enhanced mass-transfer rates because of the flow through the porous scaffolds.

Salient Features/Requirements of Perfusion Bioreactors:

i) The fluid/media should flow through the scaffold rather than around the scaffold.
ii) The fluid flow should be continuous throughout the time of the operation. Effective control systems should be present to control the flow.
iii) It should be sterilized through autoclaving or ethylene oxide sterilization throughout the time of operation to avoid any sort of contamination.
iv) The system should be simple and convi to operate to reduce possible errors.

Advantages of perfusion bioreactors over other bioreactors:

i) Requirement of minimal manpower because of easy and automated operation
ii) More productivity due to continuous and consistent operation
iii) It provides an enclosed system which is void of contamination.
iv) The flow of media solution can be easily controlled. Also the fluid pathways simulate the blood flow in vivo interstitial tissues.
v) More than one cell-scaffold construct can be cultured in one go.

CHAPTER 2

LITERATURE REVIEW

BRIEF REVIEW:

Currently, Perfusion Bioreactors have been widely used for regenerating bone tissues. Designs described by Bancroft et al [2], Jansen et al [3], Luanfeng et al [4], Grayson et al [5] mention the use of shear stress due to perfusion flow to articulate the body environment. But application of shear stress is not a sufficient criterion to regenerate efficient constructs for bone-ligament interfaces. The design described by Bancroft et al [2] consists of six chambers machined into one block of Plexiglas, each of the six chambers comprises two neoprene O-rings and a cassette in between which houses the scaffold. Media is pumped from a common reservoir into these 6 independent chambers with the help of a multi-channel pump. This flow system ensures delivery of accurate and controlled flow rates as well as a heightened consistency.

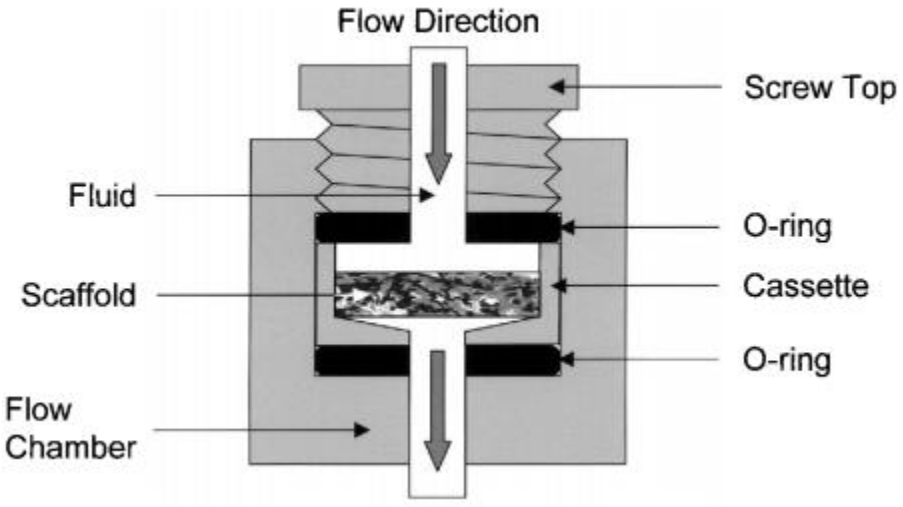

FIGURE 2.2 *One of six flow perfusion chambers as described in Bancroft et al [2]. Media is perfused through the scaffold because of the vertical flow of media which is perpendicular to the scaffold.*

The design described by Hohlrieder et al [1] shows a modern bioreactor which simulates both mechanical and biochemical environment for reconstruction of ACL. The bioreactor has capability to apply biaxial mechanical strains to scaffolds, axial for tensile forces and rotational for compressive forces. Wayne et al [6] describes another bioreactor which does not use perfusion flow, but comprises biaxial loading system for tension and compression. Analysis of the results of both systems is positive. Results of the former showed that the fibroblast adhered to

and covered the fibers and hence, the tissue construct mimicked the human ACL. Results of the latter showed that mechanical loading gave preferred compressive and tensile resistance by increasing extracellular products and also amount of nutrients to the interior parts of the bone-ligament constructs.

Not many Bioreactors have efficiently regenerated bone-ligament interfaces. So this is where the proposed bioreactor comes in. It is a Perfusion Bioreactor which has completely independent setups for application of tension and compression. Since the systems are independent, tensile and compressive force can be applied simultaneously. The proposed bioreactor intends to combine the advantages of three bioreactors (Hohlreider et al [1], Bancroft et al [2] and Wayne et al [6]); it employs perfusion flow, has better clamping mechanism and has biaxial mechanical stimulation setups.

CHAPTER 3

MATERIALS AND METHODS

3.1 COMPUTER-AIDED DESIGN OF PERFUSION BIOREACTOR:

3.1.1 *In silico* Design:

All designs were made using the Autodesk Inventor Software. Listed below are the components present in the design:

i) Lower Compartment:
ii) Upper Compartment
iii) Compartment Lid
iv) Syringe Piston – Bone support

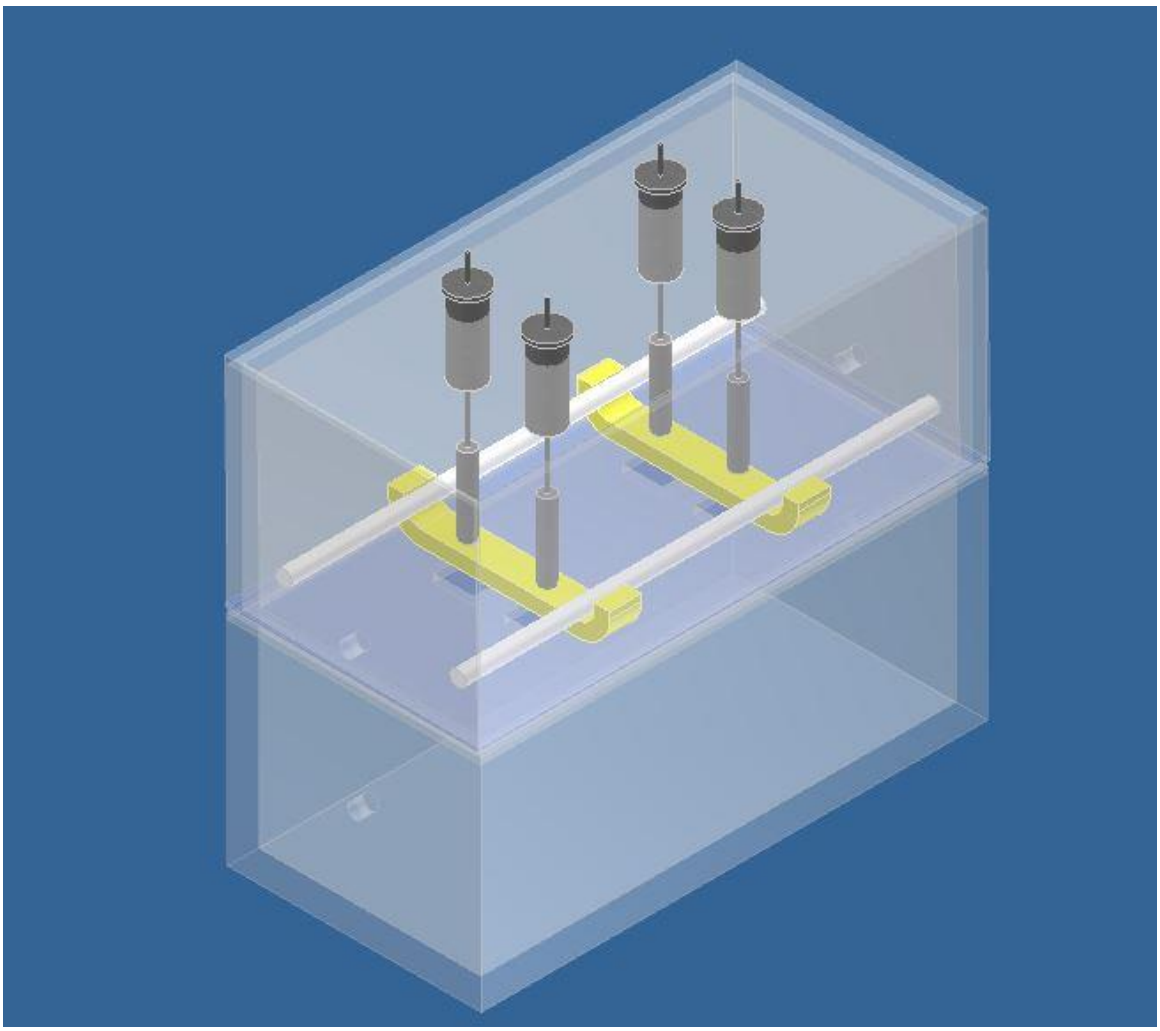

FIGURE 3.1 *Assembled Reactor Design*

3.1.2 The Reactor Components in Brief:

Lower Compartment:

The Lower Compartment was solely meant for controlled flow of water. It contained one inlet and one outlet for water. Water was driven from the inlet by means of a water pump and dumped from the outlet to a single water tank.

Upper Compartment:

The Upper Compartment was dimensionally same to the Lower Compartment and was kept on top of it. The difference being that the upper compartment consisted of square holes at its base which was covered by a PDMS Sheet. The Upper compartment was meant for housing the culture. It had a media inlet and outlet ports and two clamped pipes on two sides for tensile setup.

Compartment Lid:

Compartment Lid was dimensionally same to the base of any of the two compartments. It comprised round holes through which syringes were inserted for the compression setup. It was kept over the upper compartment, and easily separable from the same.

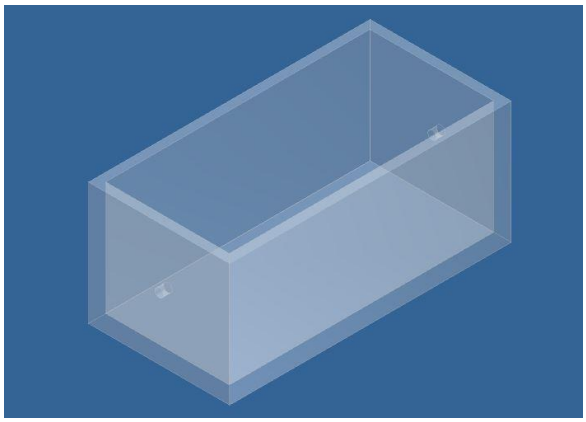

FIGURE 3.2 *Lower Compartment Design*

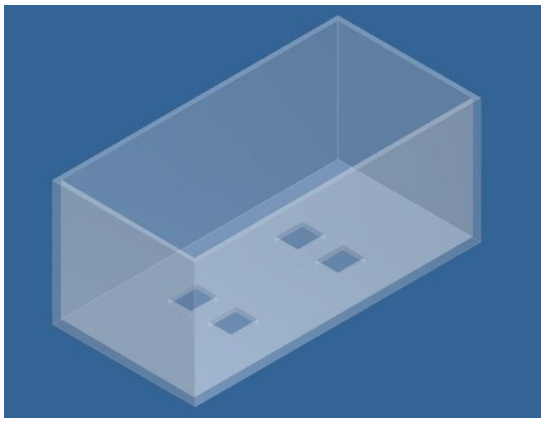

FIGURE 3.3 *Upper Compartment Design*

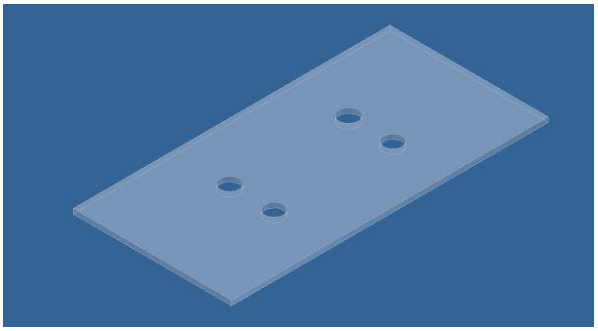

FIGURE 3.4 *Lid design with holes for inserting syringes*

FIGURE 3.5 *Syringe Piston driven bone support design*

3.2 FABRICATION PROCESS:

Based on the preliminary software design, fabrication of the bioreactor was initiated. The fabrication was done part-wise and then assembled to construct the bioreactor.

3.2.1 Reactor Vessel: Lower and Upper Compartments:

For Reactor Vessel, 4 Safety Eyewear Boxes were ordered from Tarsons Products Pvt. Ltd, Product Code 800030. As per the specifications in the Tarsons Catalog, each Safety Eyewear Box was of the dimensions 32cm X 16cm X 14cm. The height was actually 7.5cm with one side elevated to 6.5cm as shown in **FIGURE 3.6** below. The Safety Eyewear Box was considered fit for the bioreactor compartments because of its transparency and material composition, PMMA (Polymethyl-methacrylate). PMMA is biocompatible polymer, easily autoclavable and also resists ethelene oxide sterilization.

The elevated height was of no specific use to the bioreactor. So, it was cut-off using Jig-saw. The results are shown in **FIGURE 3.7**. The rough surface after cutting was blasted by sand paper.

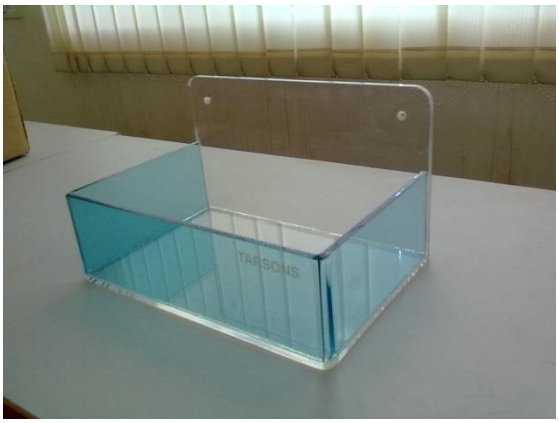

FIGURE 3.6 *Reactor vessel when procured*

FIGURE 3.7 *Reactor vessel when elevated height cut-off*

All the processes mentioned above were carried out for both upper and lower compartments. In lower compartment, Drilling Machine was used to drill two 7mm holes for inlets and outlet. In upper compartment, four rectangular holes were drilled on the base plate using Drilling Machine and Jig-saw. Then, using Wireless Automatic Drilling Machine and a tool of 20mm diameter, four holes were drilled, two each on the east and the west faces. **FIGURE 3.8** shows the upper compartment after all these operations.

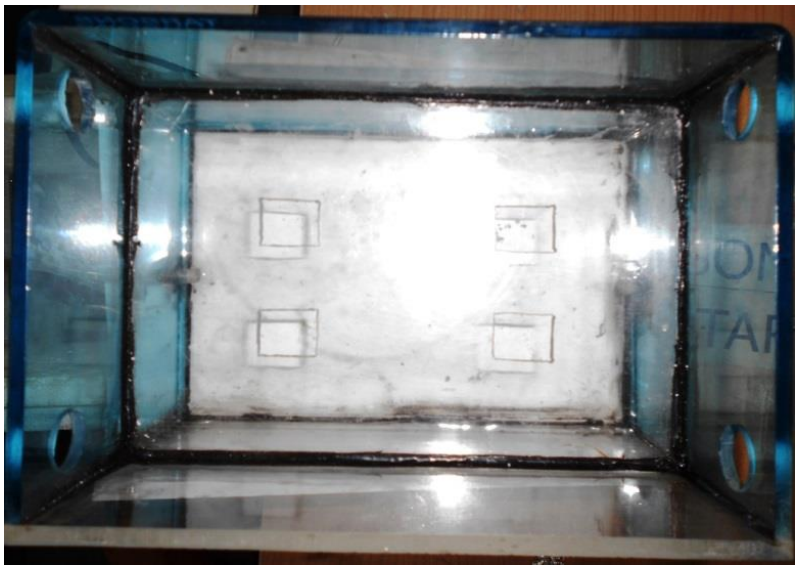

FIGURE 3.8 *Upper Compartment Top View (The square holes on its base and circular holes on its east and west faces are visible)*

Silicon gel (black) was used for sealing the ends and edges of the compartments to avoid any leakage. Silicon gel is biocompatible, as well as stable and would not harm the culture.

Araldite was used for fixing or gluing any surfaces. Since araldite is not biocompatible, after the drying of Araldite layer, a layer of Silicon gel was applied over it everywhere in the upper compartment so that the media or the cells would not get contaminated by Araldite.

3.2.2 Compartment Lid:

For dimensional similarity and same biocompatibility profile, the compartment lid was made from the base of an extra 3rd Safety Eyewear Box. It was cut-off using Jig-saw. Diameter of a 12 ml syringe was measured using Vernier Calipers and found out to be 16mm. Then, again using Wireless Automatic Drilling Machine and a tool of 16mm diameter, 4 holes were drilled in the compartment lid for inserting the syringes. **FIGURE 3.9** shows the compartment lid.

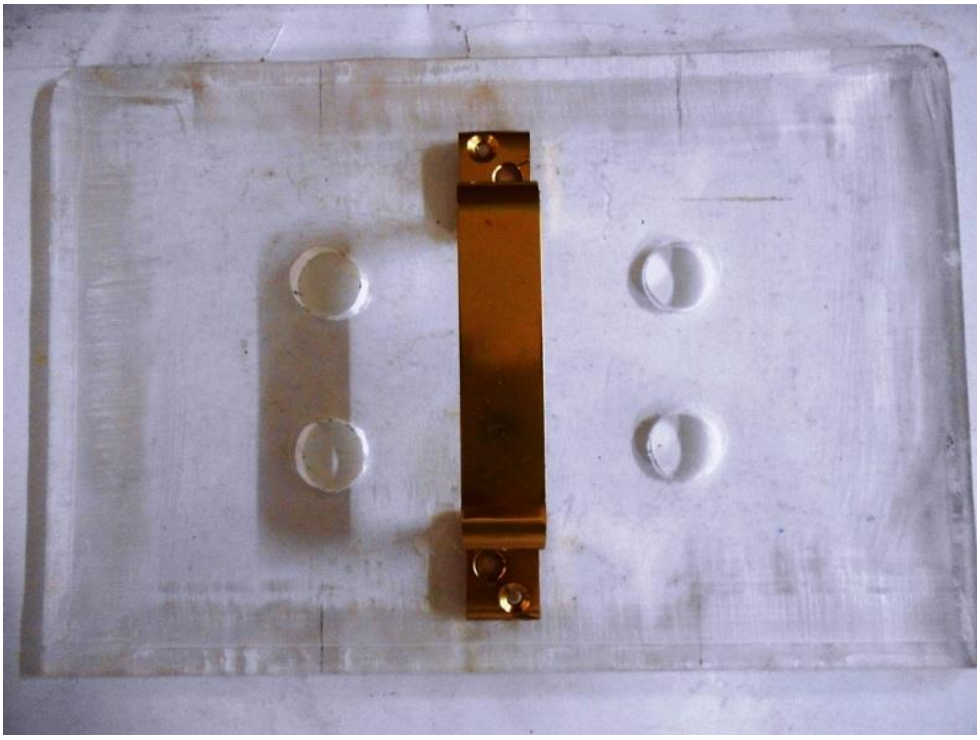

FIGURE 3.9 *Compartment Lid with aluminum handle (Circular holes for inserting syringes)*

A door handle made up of Aluminum was used for the handle of the lid. Fixing it to the lid using Araldite was preferred over drilling on the lid.

3.2.3 Compression Setup:

3.2.3a Components and Construction:

Compression Setup was fabricated from Silicone sheet, four 12 ml syringes, two 5 ml syringes, syringe pump and pumice stone.

PDMS Sheet was not available, so Silicone sheet was used. Silicone is a large family of compounds also containing PDMS. It has properties like elasticity, high biocompatibility, heat resistance, etc. which makes it significantly useful in the perfusion bioreactor. The Silicone sheet ordered was 30cm X 30cm in dimension and 3mm thickness. The 3 mm thickness was not small enough for use in the compression setup. To reduce the thickness, first the sheet was cut into smaller pieces (11cm X 6cm) covering two holes of the upper compartment base and then it was scraped off using surgical blades as shown in **FIGURE 3.10** until a near-uniform thickness of 1.6mm was obtained. Thickness was measured using Digital Vernier Calipers. The Silicone sheet was placed and glued over the holes in the base plate using Araldite first, and then by Silicon gel.

Four 12 ml syringes were cut using heated surgical blade (**FIGURES 3.11** and **3.12**) to get a reduced length of 4cm. Two 5ml syringes were cut in the same manner to get four tubes each of length 1 cm.

Pumice Stone was cut using paper cutter into 4 identical pieces (**FIGURE 3.13**) each with 3cm X 2 cm base (size equivalent to the hole in the base plate of Upper compartment) and height 2cm. Pumice stone was used for its porous nature, so that if cells grow, it can diffuse into the porous structure. Pumice as such is not proved to be biocompatible, but it is used in small amounts in toothpastes as well as it is used in cosmetics for rubbing of skin.

The smaller cut syringe was glued to the top face of the pumice stone first using Araldite and then covered by Silicon gel. Then, the upper face of smaller syringe was inserted into the bigger cut syringe and attached to the piston of the bigger syringe such that movement of the 12 ml syringe piston would lead to movement of the whole setup including pumice stone and smaller syringe cut portion. Three other instances of such structures were created to make a total of four and the bigger syringe was inserted into the circular hole in the compartment lid. The final setup for compression is shown below in **FIGURE 3.14**.

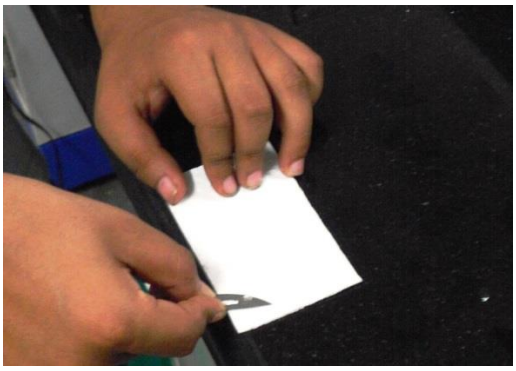

FIGURE 3.10 *Scrapping off the Silicone Sheet using Surgical Blade*

FIGURE 3.11 *Burning a Surgical Blade (Right)*

FIGURE 3.12 *Cutting the syringe*

FIGURE 3.13 *Cutting pumice stone with paper cutter*

FIGURE 3.14 *The Syringe driven Compression arrangement (Three other instances of the same design were employed)*

3.2.3b Working:

Working of the compression setup consisted of two parts; lower and upper.

Lower part was done in lower compartment. Water was forced in through the inlet using a cooler motor. The outlet was plugged shut and water level was allowed to rise. After completely filling the lower compartment, the water level rose into the holes and finally came into the contact with the Silicone sheet above. Due to the elastic nature of the Silicone sheet, it was protruded upwards because of the force of rising water. Hence, the cell monolayer along with scaffold present just above the Silicone sheet was imparted force from below.

Other half of the compression setup was done in the upper compartment and the lid. The syringe-pumice stone arrangement shown in **FIGURE 3.14** was adjusted as such that the lower face of the pumice stone exactly touched the cell monolayer along with scaffold present just below it. Then, for compression, a syringe pump was kept vertically, which drove all the four syringes downwards. Hence, the scaffold was imparted uniaxial bi-directional compression in the stem-cell compartment of the scaffold which was expected to develop into enthesis.

3.2.4 Tension Setup:

3.2.4a Components and Construction:

The Tension setup consisted of two polymer pipes, clamps, nuts and bolts, and four stepper motors.

Polymer pipes of material Polyvinyl Chloride (PVC) were used. Stainless Steel was not used because of the high weight of the SS pipes. Titanium was not used for its high price. The pipe was 20mm in diameter, and 1.5mm in thickness. The pipe was cut into two smaller pipes of length 26cm (slightly higher than the reactor vessel length 23.5cm) using hack saw blade as shown in **FIGURE 3.15**.

FIGURE 3.15 *Cutting pipe using hack saw blade*

Clamps were made out of a bigger pipe, of same material, whose outer diameter is 27mm and inner diameter is 19mm (almost equivalent to the diameter of the smaller pipe). It was cut in pieces of 5.6 cm (greater than the width of the scaffold) and halved using hack saw blade. Scraping was done to ensure proper fitting of the bigger pipe over the smaller pipe. 4 such clamps were used, 2 on each pipes. **FIGURE 3.16** shows an instance of the clamping system.

FIGURE 3.16 *Instance of Clamping System using nut-bolts and polymer pipes*

Nuts and bolts were used to hold the clamps in place. The material used was brass. Two nuts and bolts were each used for one clamp, for a total of 8.

Stepper Motors were used to rotate the pipes. Rotors of Motors were fixed at the ends of the pipes such that motion of the motors would lead to rotation of the pipes.

3.2.4b Working:

4 stepper motors were used with two of them moving in clockwise direction and two of them in anticlockwise direction. This motion was supposed to drive the two pipes in opposing motions, one clockwise, another anti-clockwise and vice-versa. This drove both the pipes either outward or either inward depending on the direction. Outward Movement meant the scaffold would be stretched, and inward movement meant the scaffold would be relaxed. A schematic showing the outward movement is represented in **FIGURE 3.17.**

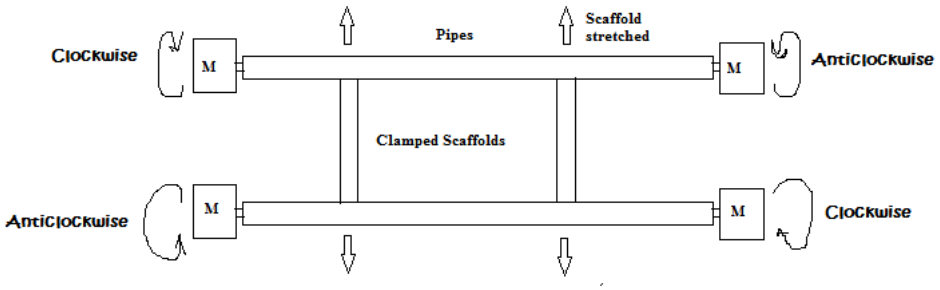

FIGURE 3.17 *Schematic showing imparting of tensile stress to scaffolds*

3.2.4c Stepper Motor Driver Circuit:

Stepper Motor Basic Theory is presented in Appendix. The Stepper Motor controller circuit is shown in **FIGURE 3.18**. The motor was driven by Darlington pair ULN2003 (Pin Diagram: **FIGURE 3.21**) coupled with Microcontroller AT89C52, commonly known as 8052 Microcontroller (Pin Diagram **FIGURE 3.19**). The circuit shown was modified a bit using Four-way switch or Intermediate switch to reverse the slots of the connecting wires so that the stepper motor rotated in reverse direction. The full circuit is shown in **FIGURE 3.23**).

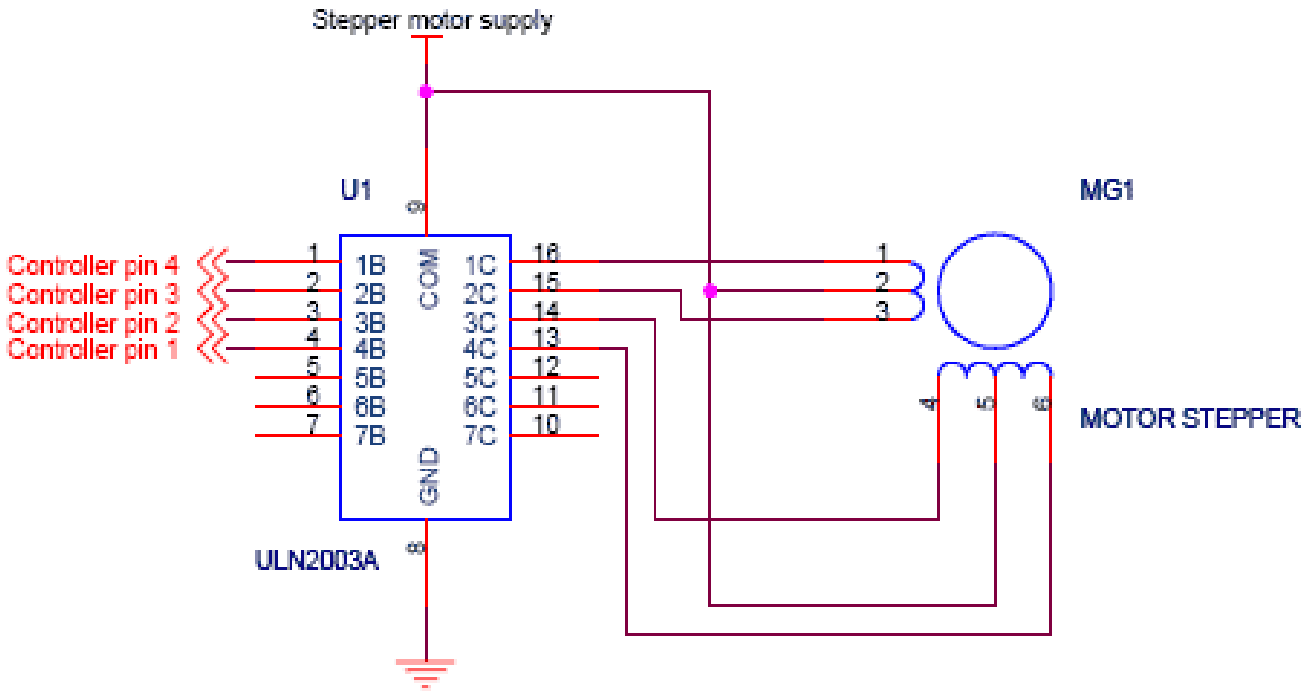

FIGURE 3.18 *Circuit Setup for driving motor using ULN2003A and Microcontroller*

Ref: *http://www.8051projects.net/stepper-motor-interfacing/stepper-motor-connections.php*

Microcontroller AT89C52, manufactured by Atmel Corporation, is a cheap, high performance, low-power, CMOS 8-bit microprocessor with 8 Kb ROM (Flash programmable and erasable read only memory). AT89C52 and AT89C51 are compatible and comparable twins of each other, both manufactured by Atmel. It is highly-flexible and cost-effective solution to many embedded control applications.

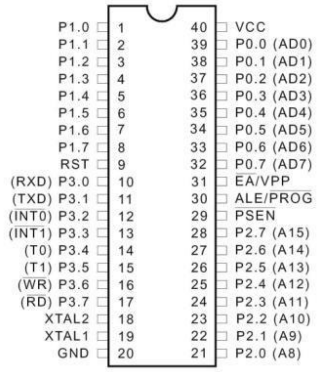

FIGURE 3.19 *Pin Diagram of 40 pin AT89C52 Microcontroller (P1.0 to p1.3 are used as output ports to connect to circuit in FIGURE***3.18***, VCC is connected to 5V Supply, EAVPP Pin no. 31 was also connected to 5V Supply because internal program is being executed*

AT89C52 or 8052 microcontroller was programmed using Superprogrammer 580-U and Superpro software. First of all, the C code was written for stepper motor drive, it was then compiled in Keil's µVision IDE for 8052 and hence a .HEX file was generated. After the generation of the .hex file, the file was dumped onto the 8052 microcontroller using the Superpro 580-U. FIGURE**3.20** shows the Keil's µVision IDE Environment as well as the code used.

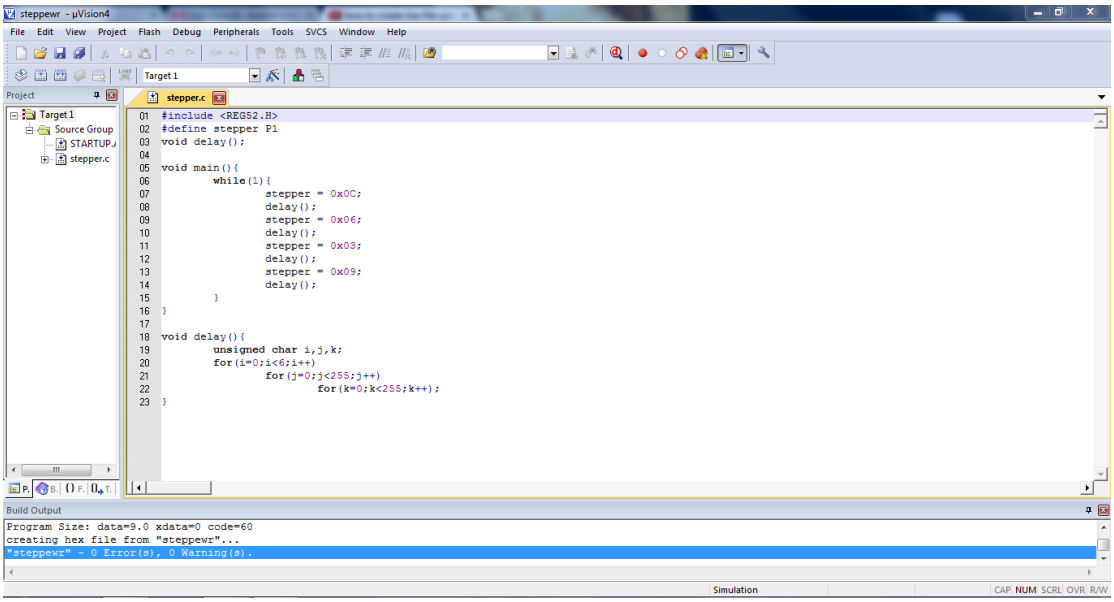

FIGURE 3.20 *Keil's µVision IDE Environment and the code written for 8052. In the bottom, it can be seen that an output is printed as "Creating hex file from "stepper"... which indicated successful compilation of .hex file to be dumped into the 8052 microcontroller.*

Darlington pairs are used to drive inductive loads including stepper motors. Darlington Pair IC ULN2003A is a high-voltage high-current Darlington transistor array. It consists of seven NPN Darlington pairs that feature high voltage outputs with common-cathode clamp diodes for switching inductive loads. The collector-current rating of a single Darlington pair is 500 mA. The Darlington pairs can be paralleled for higher current capability. FIGURE**3.22** shows the logic circuit of ULN2003A.

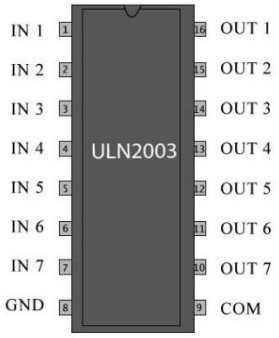

FIGURE 3.21 *ULN2003 Pin diagram (+12 V Supply given at COM terminal no. 9)*

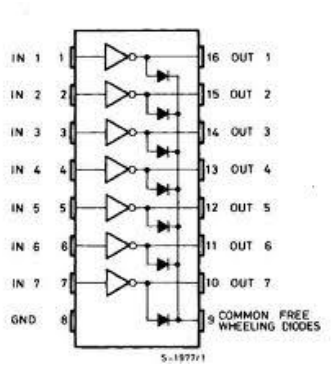

FIGURE 3.22 *ULN2003 Logic Diagram*

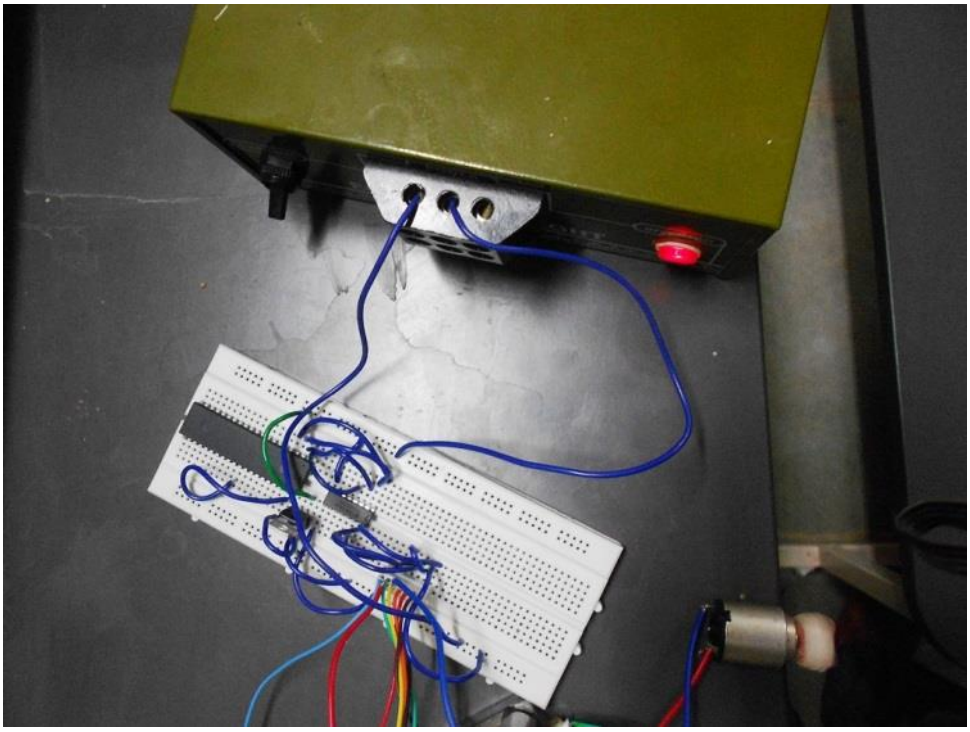

FIGURE 3.23 *Motor Drive Circuit*

3.2.4d Final Assembly:

Hence, the stepper motor rotors were fixed onto the PVC pipe using duct tapes and all possible mechanisms to make the pipe move along with the rotor. When the power was switched on, the scaffold was imparted tensile stress due to the outward motion of the pipes. Using the reverse switch, the scaffold was relaxed.

3.3 Sensors:

Sensors are integral parts of any bioreactor. They impart information on the environmental conditions inside the bioreactor such as temperature, pressure, dissolved oxygen, etc.

In the bioreactor we designed, two sensors were used, one was temperature sensor to monitor the temperature, and another was force sensor to monitor the force that the rising water level exerted on the stem-cell compartment. DO and CO_2 sensors were not used because finally, the bioreactor would be kept inside the CO_2 incubator for culture.

3.3.1 Temperature Sensor:

Temperature Sensor used was LM35 manufactured by National Semiconductors. LM35 are precision-integrated-circuit temperature sensors, where output voltage is linearly proportional to the Celsius (Centigrade) temperature.

Salient Features of LM35:

i) Calibrated directly in ^{0}C (Celsius)
ii) Linear Response, Sensitivity is 10mV/^{0}C
iii) Accuracy = 0.5^{0}C (at 25^{0}C)
iv) Range = -55^{0}C to 156^{0}C
v) Low Self-Heating
vi) Non-linearity only ±1/4^{0}C
vii) Sensing circuit easy to build (easily available components)

FIGURE 3.24 *Pin diagram of LM35*

FIGURE 3.24 shows pin diagram of LM35 temperature sensor. It takes 5V as input supply, and gives output in voltage. This voltage (V_0) is directly related to the temperature by the following equation:

$$V_0 = f(T) = 10T$$

Where, V_0 = Output of LM35 in mV

T = Temperature in ^{0}C.

The temperature output was then coupled with Arduino Uno Board and the temperature was displayed in the 16 bit LCD Display Panel (JHD 162A) as shown in FIGURE **3.26**.

Arduino Uno is a mini-size portable microcontroller board manufactured by Arduino Co. FIGURE **3.25** shows the Arduino Uno board. Significant features of this microcontroller include six analog input ports (A0 – A5), a 5V and 3.3V DC power supply ports and 14 Digital output ports. Arduino Uno can be easily programmed in basic C or C++ language. Arduino Co. itself has developed softwares, based on C or C++ as a platform, to program the microcontroller. Interfacing with any computer is very easy using the USB cord.

FIGURE 3.25 *Arduino Uno by Arduino Co*

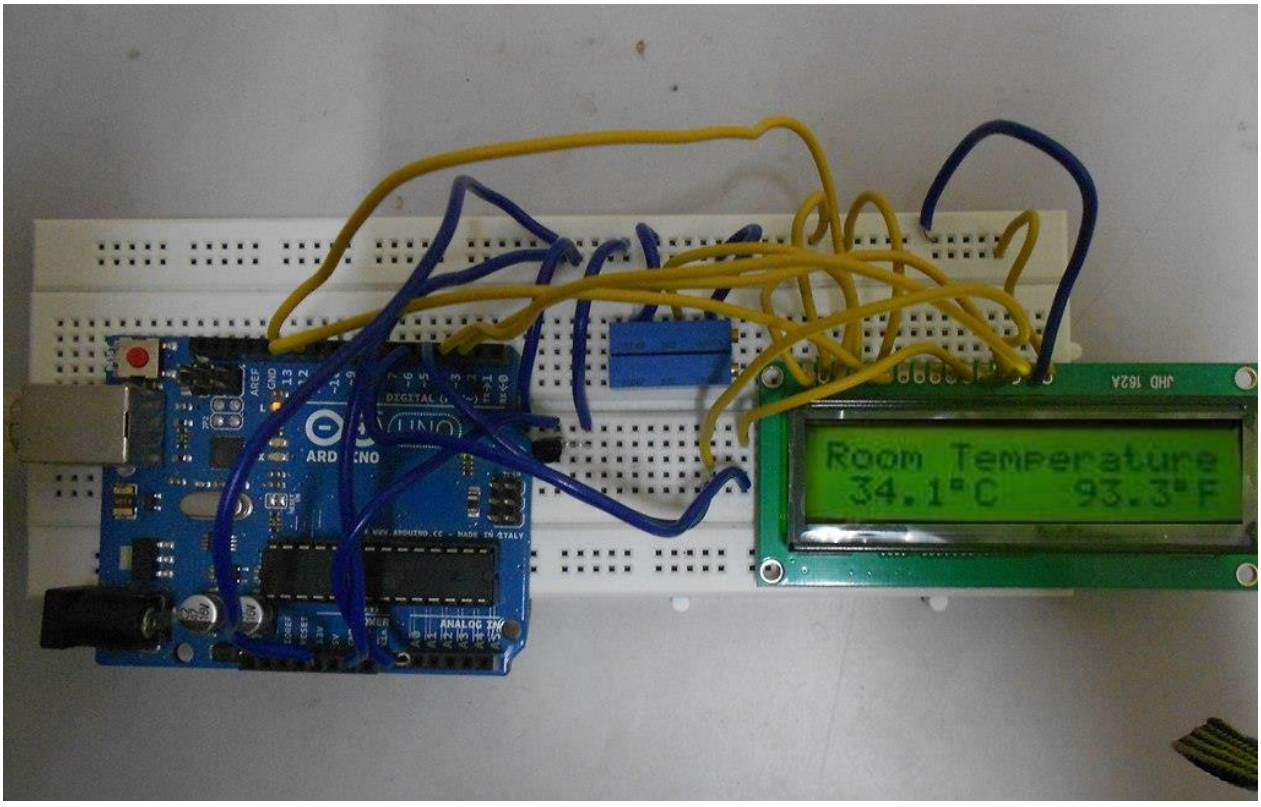

FIGURE 3.26 *Printing temperature through 16bit LCD Display*

3.3.2 Force Sensor:

Force Sensor used was Force Sensitive Resister (FSR 400 Series Round Force Sensing Resister) by Interlink Electronics which is shown in FIGURE**3.27**. FSR consists of a robust polymer thick film (PTF) that exhibit a decrease in resistance with increase in force applied to the surface of the sensor. It is small, just around 4cm (1.75") in length but has certain characteristics listed as follows due to which it is popular in the commercial market.

Salient Features of FSR Small:

i) 4 mm diameter active sensing area

ii) Resistance decreases with increasing force. When no force is applied, the resistance is around 10MΩ. It decreased to 2.5KΩ when maximum force is applied.

iii) Sensitivity Range is 0.1N to 10N.

iv) Actuation force can be as low as 2 gm. weight.

FIGURE 3.27 *FSR Small top view and bottom view*

Resistance – Force Graph:

The Resistance – Force Graph as shown in the FSR Datasheet is given below in FIGURE**3.29**. The graph is double log graph i.e. log-log graph and the relation shown is almost a straight line. An equation was deduced from the graph so as to relate Resistance with Force.

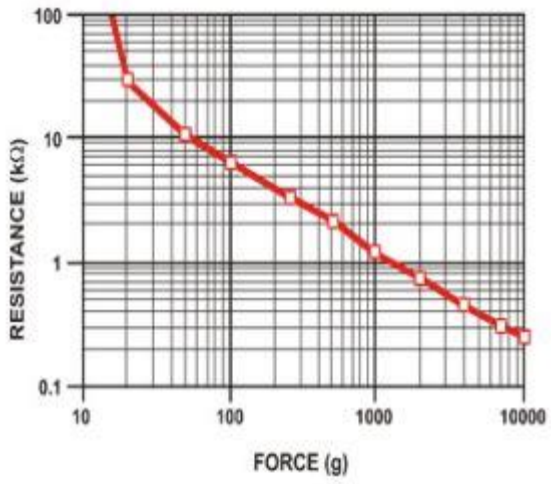

FIGURE 3.28 *Force-Resistance curve*

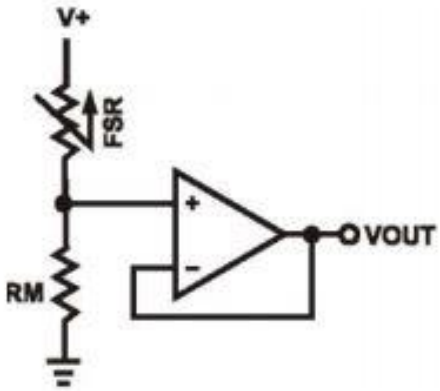

FIGURE 3.29 *Voltage-Divider Circuit*

General equation for straight line on a log-log graph:

$$\frac{\log_{10}(R) - \log_{10}(R_1)}{\log_{10}(R_2) - \log_{10}(R_1)} = \frac{\log_{10}(F) - \log_{10}(F_1)}{\log_{10}(F_2) - \log_{10}(F_1)}$$

From this equation, and the graph points, the relation between Force and Resistance was deduced to be as follows:

$$\log_{10}(R) + 0.6826 \log_{10}(F) = 3.14336 \ \{\log_{10}(R) \leq 30, \log_{10}(F) \geq 20\}$$

$$\log_{10}(R) + 4.322 \log_{10}(F) = 7.1 \ \{\log_{10}(R) > 30, \log_{10}(F) < 20\}$$

P.S. All R is in KΩ and all F is in grams.

Then, the FSR was kept in a voltage divider circuit as shown above in FIGURE**3.30**. Again, the relationship between output and the FSR Resistance was deduced to be as follows:

$$R_{FSR} = (V_S/V_{OUT} - 1) * R_m$$

Where, R_m and R_{FSR} are resistances in KΩ

V_S = 5V and V_{OUT} = Output voltage.

The V_{OUT} terminal was again interfaced with Arduino Uno as shown in FIGURE**3.31** and force was calculated and displayed.

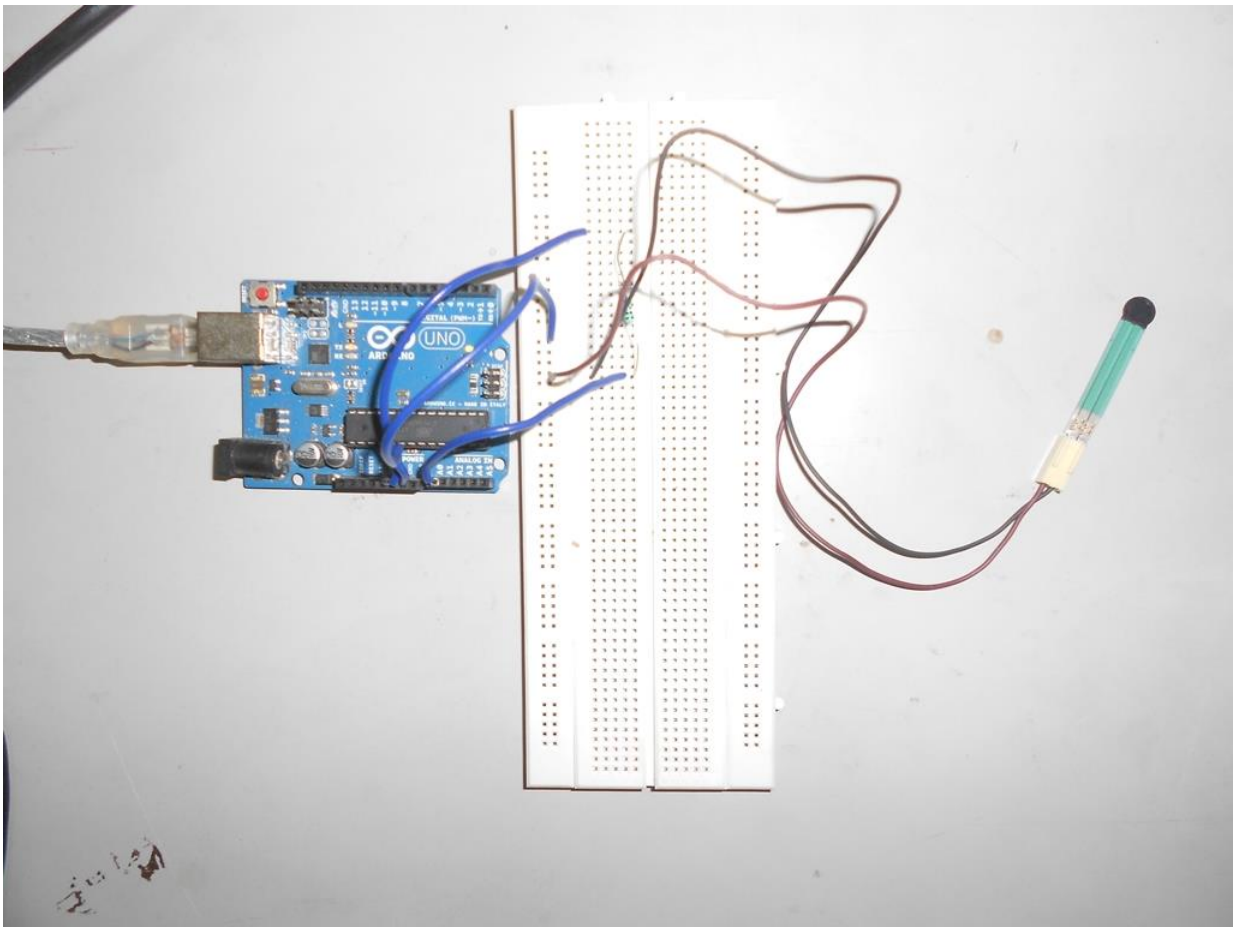

FIGURE 3.30 *Interfacing Force sensor with Arduino*

CHAPTER 4

RESULTS & DISCUSSION

4. RESULTS & DISCUSSION:

A Perfusion Bioreactor system was fabricated as shown in Figures **4.1** and **4.2** below.

The whole Perfusion Bioreactor system was found to be air-tight, with no leakage of water or media. The Clamping system was efficient in holding the scaffolds tight and properly. The Compartment Lid was properly attached to the reactor vessel.

Stepper Motors chosen were high torque and high supply stepper motors which could not be driven. But, the driver circuit was successful in driving four low torque DC motors, two in anticlockwise and two in clockwise direction simultaneously, and the directions could be interchanged. The demonstration using the low torque DC motors was done only to prove that the motor drive circuits are indeed capable of driving more than one motor in synchronous motion.

Cooler Motor was used to drive water in the lower compartment. The Rising water level indeed led to the bulging up of Silicone sheet and hence, the scaffold right above it. Compression was duly measured using FSR Sensors coupled with Arduino Uno. But the response of the FSR Sensing was slow and not highly sensitive. The cooler motor was immediately switched off after the bulging was observed. It was done so as to ensure that the Silicone sheet would not be torn apart because of excessive pressure being applied to it. The Force was calculated and duly displayed in the LCD Display Panel JHD-162A.

Temperature was duly monitored in real time as well with the help of LM35 Sensor coupled with Arduino Uno and displayed in the same LCD Display Panel. If the temperature was found to be too high (which is harmful for culture), cooling of the bioreactor should be done.

Biocompatibility of the whole system was ensured so that the cell culture would not be contaminated. Reactor vessels and Silicone sheets easily resist autoclave as well as ethelene oxide sterilization. Electronic components are supposed to be sterilized using Gamma ray sterilization.

Hence, after analyzing the results, following improvements were suggested:

i) Incorporation of high supply Stepper motor drivers for the high torque stepper motors

ii) Use of Polypropylene pipes instead of PVC Pipes because of the biocompatibility of Polypropylene

iii) Use of a Proper water pump instead of cooler motor

iv) Use of Syringe pump to drive the syringe supports from above for actual bi-directional uniaxial compression

v) Use of thinner Silicone sheets

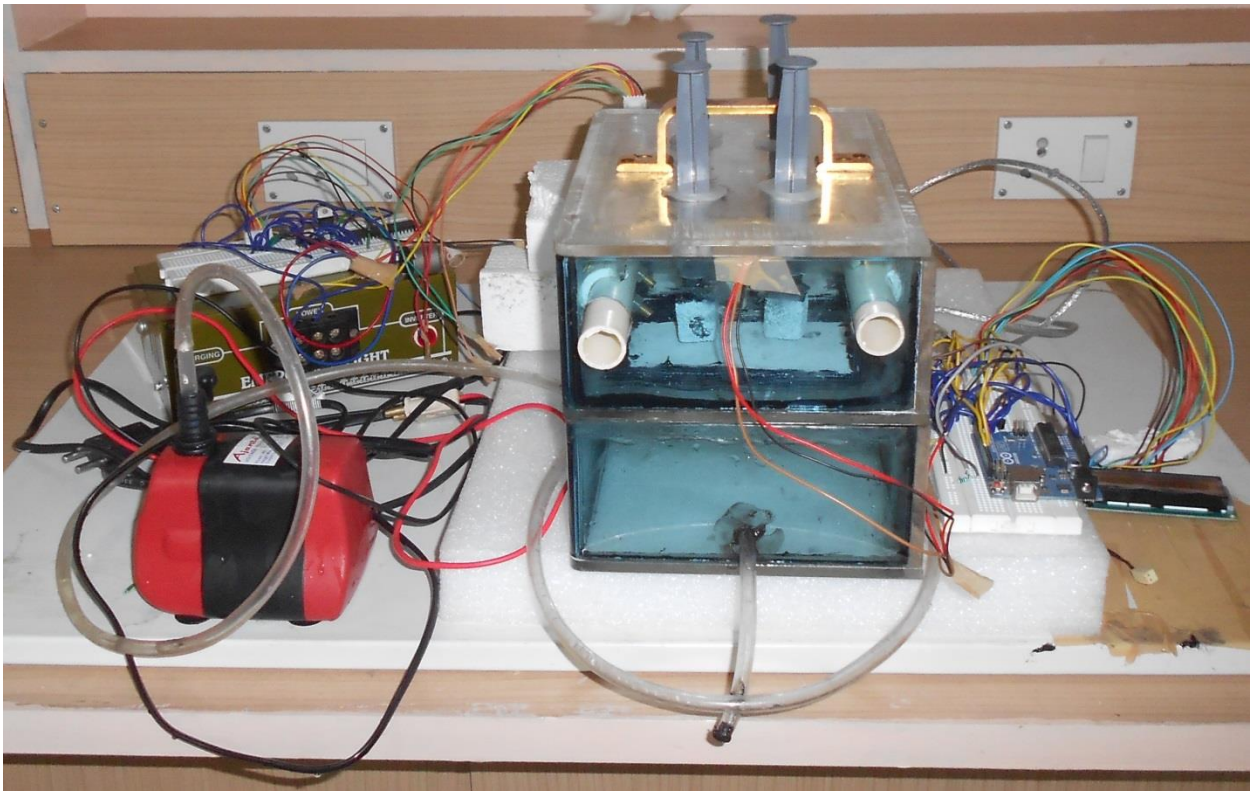

FIGURE 4.1 *Perfusion Bioreactor System (Front View)*

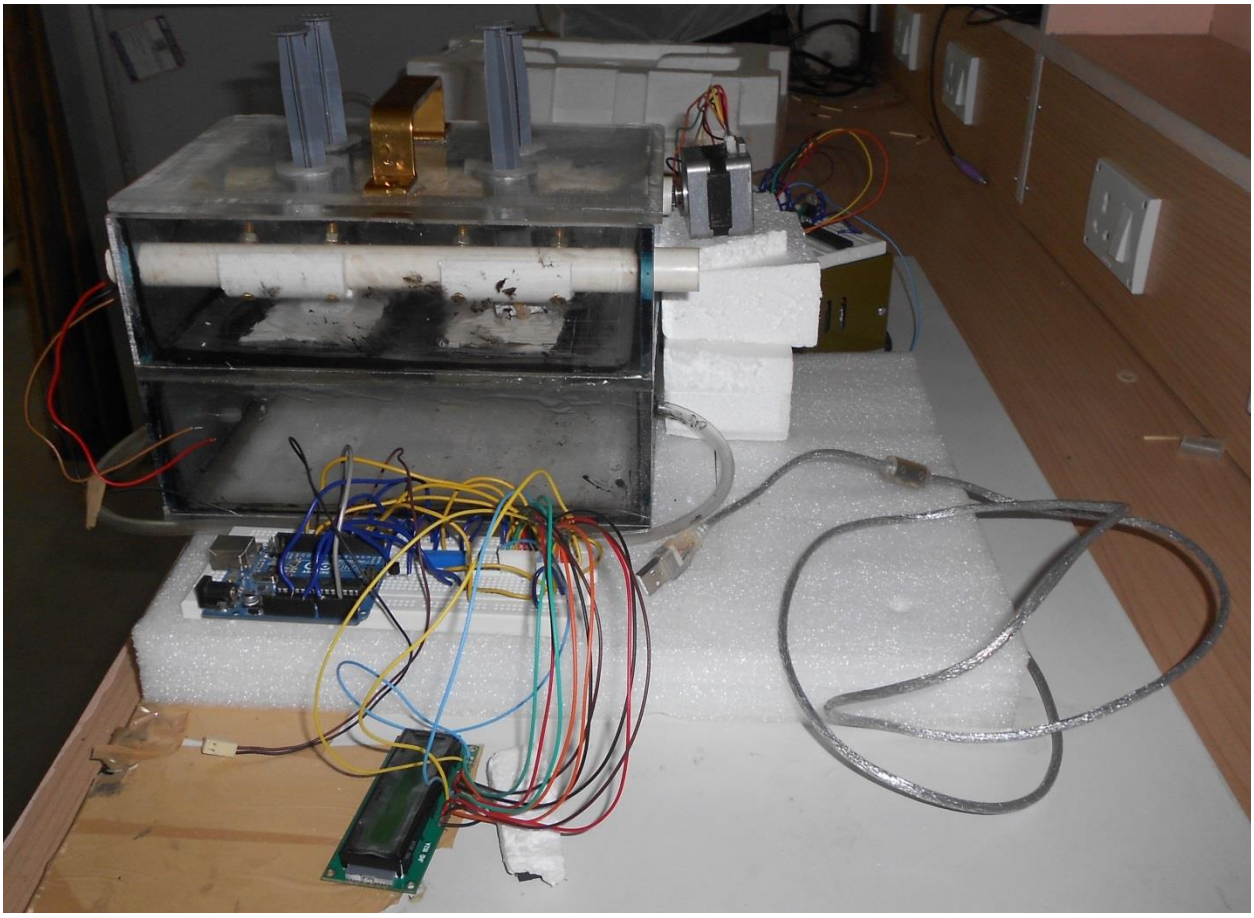

FIGURE 4.2 *Perfusion Bioreactor System (Right Side View)*

CHAPTER 5

CONCLUSION & FUTURE WORK

5. CONCLUSION & FUTURE WORK:

CONCLUSION:

Hence, a perfusion bioreactor was fabricated for regenerating bone-ligament interfaces by facilitating multi-compartmental bi-axial mechanical stimulation of the cell-seeded scaffolds. The whole system was sterilizable.

FUTURE WORK:

Future Work would consist of the finishing touches to the fabrication process along with the full automation of the process. After the automation, actual cell culture will be done in the bioreactor inside the CO_2 Incubator and Collagen expression among others will be analyzed. This will be followed by facilitating perfusion flow through the scaffolds, and hence completion of a full-fledged Perfusion Bioreactor for Interface Tissue Engineering

CHAPTER 6

REFERENCE

6. REFERENCE:

Scholarly Articles:

[1] Hohlrieder et al, Bioreactor and scaffold design for the mechanical stimulation of silk based anterior cruciate ligament grafts, O. Dössel and W.C. Schlegel (Eds.): WC 2009, IFMBE Proceedings 25/X, pp. 208–210, 2009.

[2] Bancroft et al, Design of a flow perfusion bioreactor system for bone tissue engineering applications. Tissue Engineering (2003), Volume 9, Number 3

[3] Jansen et al, A perfusion bioreactor system capable of producing clinically relevant volume of tissue-engineered bone: In-vivo bone formation showing proof of concept, Biomaterials, Volume 27, Number 3, 2006.

[4] Luanfeng et al, A Single-Use Scalable Perfusion Bioreactor System, BioProcess International, June 2009.

[5] Grayson et al, Effects of Initial Seeding Density and Fluid Perfusion Rate of Formation of Tissue-Engineered Bone, Tissue Engineering Part A, Volume 14, 2008 November.

[6] Wayne et al, Bioreactor for Biaxial Mechanical Stimulation to Tissue Engineered Constructs, Journal of Biomechanical Engineering, Vol. 131, April 2009.

Datasheets:

[7] Microcontroller AT89C52 Datasheet; Website: http://www.atmel.com/Images/doc0313.pdf; DOA: 17/09/2012

[8] Darlington pair ULN2003 Datasheet; Website: http://www.doyoung.net/video/DATASHEET / PDF/ULN2003.pdf; DOA: 01/12/2012

[9] LM35 Temperature Sensor Datasheet; Website: http://pdf1.alldatasheet.com/datasheet-pdf/view/8875/ NSC/LM35DZ.html; DOA:13/12/2012

[10] Force Sensitive Resister (FSR Small) Datasheet
Website: http://dlnmh9ip6v2uc.cloudfront.net/datasheets/Sensors/ForceFlex/2010-10-26-DataSheet-FSR400-Layout2.pdf; DOA: 22/12/2012

Other References:

[11] Stepper Motor Driving Code on open source Website: http://www.8051projects.net/stepper-motor-interfacing/programming-microcontroller.php; DOA: 03/01/2013

[12] Stepper Motor Circuit Connections; Website: http://www.8051projects.net/stepper-motor-interfacing/stepper-motor-connections.php; DOA: 19/03/2013

APPENDIX

APPENDIX:

Appendix 1: Arduino Code for Temperature and Force Sensing:

```
#include <LiquidCrystal.h>          //To interface LCD Display with Arduino Board//
LiquidCrystal lcd(12,11,5,4,3,2);          //using pins 12,11,5,4,3,2 as output to LCD//
const int inPin=0;
const int forcepin=3;
void setup()
{
  // put your setup code here, to run once//
        lcd.begin(16,2);          //Initializing LCD//
}
void loop()
{
  // put your main code here, to run repeatedly//
        int value = analogRead(inPin);          //Temperature sensing part//
        lcd.setCursor(0,1);
        float millivolts = (value/1024.0)*5000;
        float celsius = millivolts/10;
        lcd.clear();
        lcd.setCursor(0,0);
        lcd.print(celsius);
        lcd.print(" Celcius");
//Force sensing part//
        int value2=analogRead(forcepin);
        double V=(value2*1024/5);          //5V is input as 1023 i.e. 2^10 bits//
```

```
double Vs=5.0,R=0.0,R1=10.0,F=0.0;
double a=0.0,b=0.0;
if (V==0.0)
{
        F=0.0;
}
else
{
        R=(Vs/V-1)*R1;
        a= (log(R))*0.434294481;          //a=log10(R)//
        if (a<=30)
        {
                b=(2.14336-a)/0.6826;
        }
        else
        {
                b=(7.1-a)/4.322;
        }
        F=exp(b/0.434294481);             //F=10^(b)//
}
lcd.setCursor(0,1);
lcd.print ("Force"); lcd.print (F);
delay(1000);          //Delay of 1000 milliseconds i.e. 1 second between readings//
}
```

THANK YOU